U.S. Patent Application
Serial No. 09/756,978

Attorney Docket No: 10582-1

Proposed Claim Amendment

1. A method of ~~alleviating a~~ inducing tumor cell death in a human patient, the method comprising

i) locally administering ~~to the tumor~~ an antigen-releasing agent to a solid tumor in the patient, whereby a tumor antigen is released from cells of the tumor;

iia) locally administering to the tumor a leukocyte attractant, whereby leukocytes are induced to infiltrate the tumor; and

iib) locally administering to the tumor interferon-gamma (IFN-g) and a second type 1 inflammatory response- (IR1-)promoting agent, whereby a type 1 inflammatory response is induced in the tumor and ~~the~~ tumor cell death is ~~alleviated~~ induced.

Analysis of Prior Data Demonstrating Efficacy of the Claimed Methods

Basis and Schematic Description of the Therapeutic Modulation fo the Tumor Inflammatory Response (TMTIR) Biotreatment

We have established that initiation of an effective inflammatory response is characterized by six events:
1) Release of antigens from altered cells at the injured site and local secretion of chemotactic factors,
2) Infiltration of the disease site by the immune cells,
3) Polarized type 1 or type 2 activation of the immune cells by the antigens released at the site,
4) Amplification of the inflammatory response over time, at least for a limited period,
5) Elimination of the diseased or pathological cells by the immune cells,
6) Conversion of activated immune cells into memory cells that can provide long-term protection against the antigen or antigen-bearing cells.

At least item 3 is necessary in order to mount an effective immune response, and items 1) and 2) can significantly improve that response.

We have defined our multi-step biotreatment based on the six events presented above required to induce a novel (intratumoral inflammation reaction) IIR and the scientific facts presented below. The TMTIR initiates and sustains an intratumoral type 1 IR in any tumor types **(see Figure 1)**.

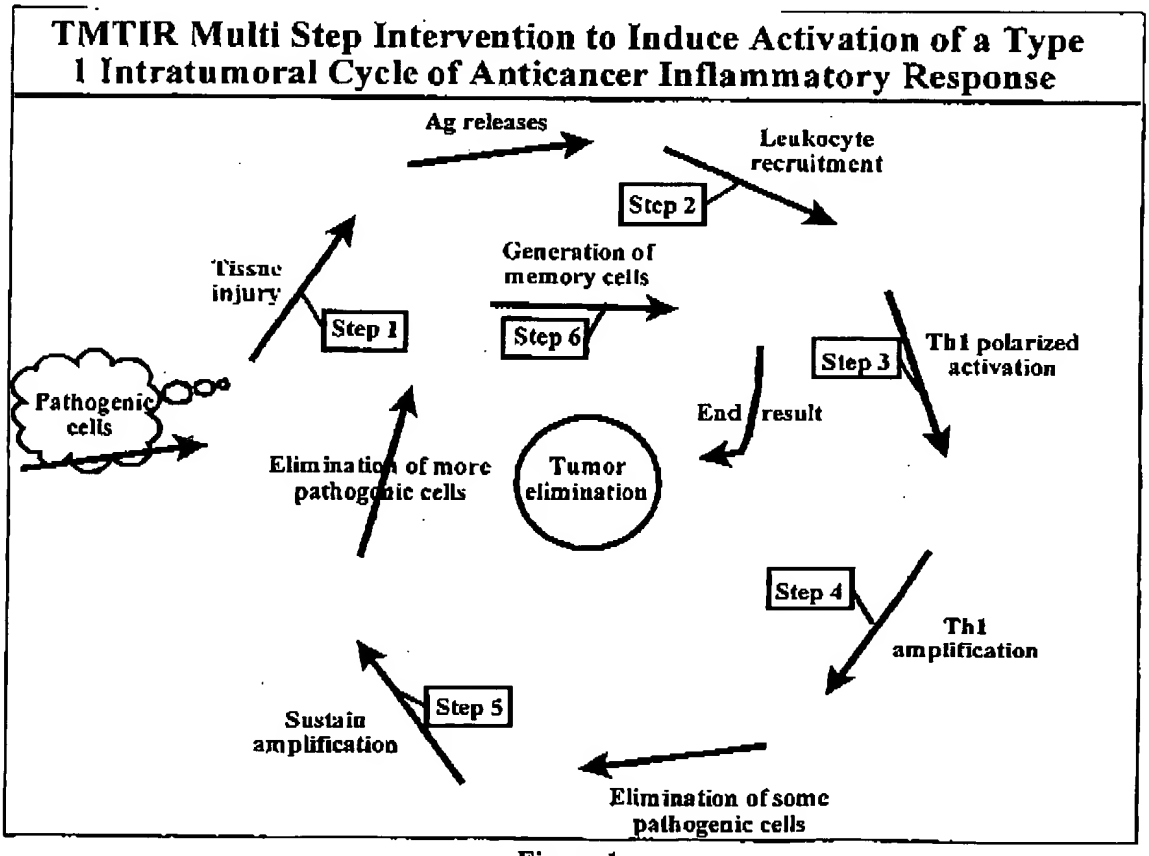

Figure 1

This method of shrinking tumors, included in the 66 claims of the TMTIR patent application, is based on the following scientific facts: **1)** Evidences for the polarization of the immune response to either type 1 or type 2. **2)** Evidence that tumors grow in presence of a type 2 intratumoral inflammation. **3)** Evidence that naturally occurring tumor regression in animals and humans caused by development of an intratumoral type 1 inflammatory response. Tumor regression are obtained also in animal models in which conditions are practiced to induce an intratumoral type 1 inflammatory response. **4)** The BCG treatment for human bladder cancer induces an intratumoral type 1 inflammation. It achieves complete remission in over 70% of patients.

1) Evidence for the polarization of the immune response to either type 1 or type 2

The Immune Response (IR) naturally polarized either into a type 1 or a type 2 function **(see Figure 2)**, depending of the antigens encountered. The type 1 IR is characterized mainly by the presence and production of the cytokine IFN-g whereas a type 2 IR is characterized mainly by the presence and production of the cytokine IL-4. The type 1 IR can exert strong cytotoxicity against cancer cells and can destroy tumors whereas the type 2 IR does not support tumor cytotoxicity, Mosmann et al. (Journal of Immunology Vol.136, Issue 7, pp. 2348-2357, 1986), Romagnani (Annu Rev Immunol Vol. 12, pp. 227-257, 1994), Romagnani (Ann Allergy Asthma Immunol. Vol. 85 (1), pp. 9-18, 2000).

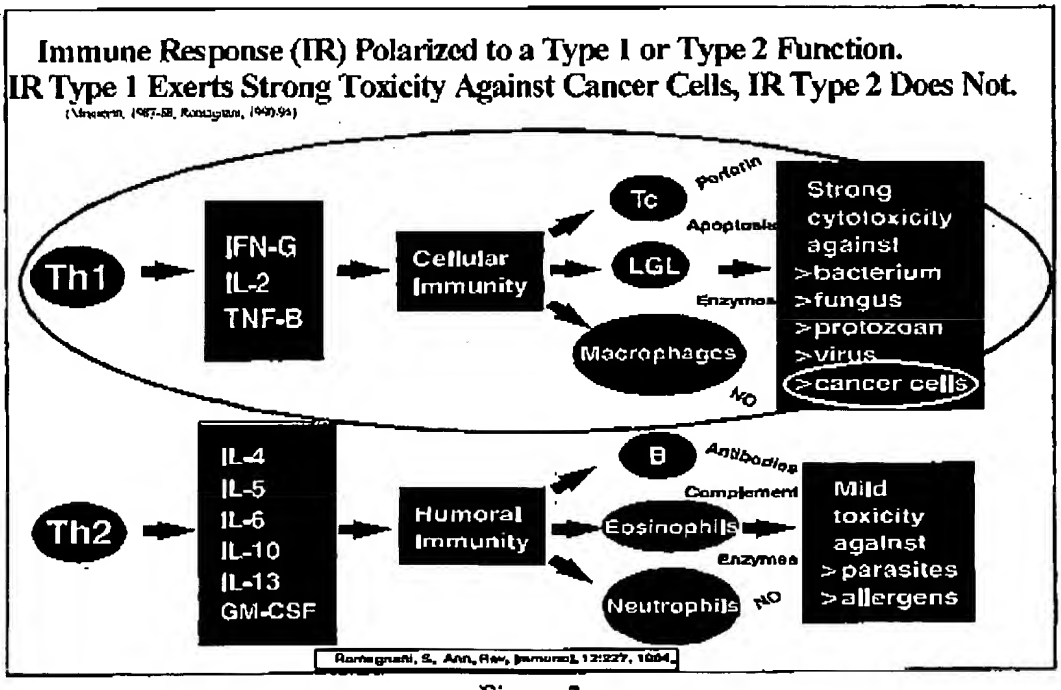

Figure 2

2) Evidence that tumors grow in presence of a type 2 intratumoral inflammation

Polarization of the intratumoral inflammation reaction (IIR) is determinant in the growth or regression outcome of a tumor. Tumors progress from a defective antitumoral inflammatory response, Roussel et al., 1996, Balkwill et al. (The lancet Vol. 357, pp. 539-545, 2001) and the defect is a type 2 IIR (ineffective against tumors). Several studies of tumor inflammation have all found that a predominant polarized Th2 IIR was present in different types of growing tumors such as lung, brain, breast, and kidney tumors, Roussel et al. 1995, 1996, Camp et al. 1996, Maeurer et al. 1995, showing the universality of the phenomenon, **(see Figure 3 for typical data).**

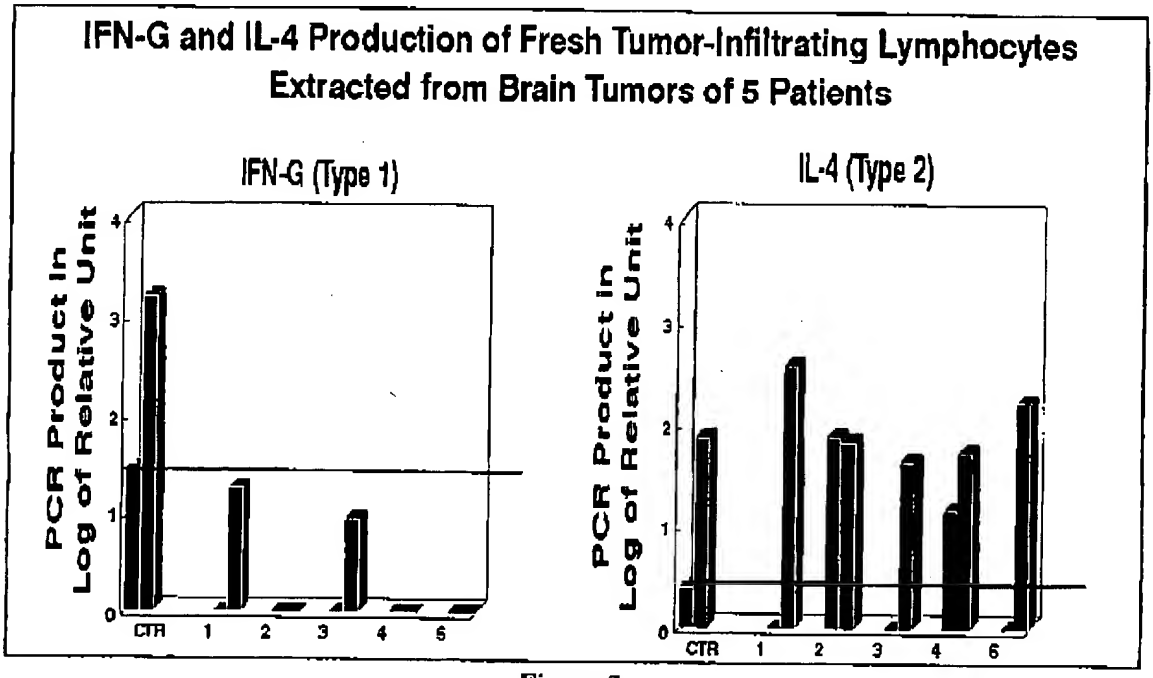

Figure 3

Analysis: Lack of interferon-gamma (IFN-G) and significant production above baseline control (transversal lines) of interleukin-4 (IL-4) by TIL (right black bars) from growing human brain tumors, indicative of a type 2 intratumoral inflammation. Data extracted from Roussel, 1996.

3) Evidence that naturally occurring tumor regression in animals and humans are caused by the development of an intratumoral type 1 inflammatory response. Tumor regression are also obtained in animal models in which conditions are practiced to induce an intratumoral type 1 inflammatory response

The influence of an IIR type 2 versus IIR type 1 on tumor outcome was studied by different scientific groups doing comparative examination of the IIR in conditions of growing versus regressing tumors. Polarized Th2 inflammatory responses were found in growing tumors whereas polarized Th1 inflammatory responses were found in regressing tumors, Kurt et al. (J. Immunol. Vol. 154(8), pp. 3969-74, 1995), Yamamura et al. (J. Clin. Invest. Vol. 91(3), pp.1005-10, 1993), Lowes et al. (J. Invest. Dermatol. Vol. 108(6), pp. 914-9, 1997).

For instance, Khar et al. 1997 **(see Figure 4)** showed in a model of spontaneous regression of AK-5 histiocytoma in syngeneic rats, that increase levels of IL-2, IFN-gamma, and TNF-alpha (three lymphokines characteristic of the type 1 response) were correlating with regression of the tumor tissue. Sustained high concentration of these type 1 cytokines in the tumor tissue correlated with induced shrinkage of tumors, indicating an active intratumoral type 1 response in regressing tumors. In another mouse model of tumor induced regression with IL-12 and cyclophosphamide (an agent potentiating cell-mediated cytotoxicity), large MCA207 tumors were completely eradicated within 4 weeks of a single intervention. Cellular and molecular analysis of the antitumoral inflammatory response at week 3 of regression revealed the presence of type 1 infiltrating T cells producing abundant IFN-gamma (type 1 IIR) and no IL-4 (the main cytokine of type 2 IIR), Tsung et al. 1998).

To test the therapeutical effect of a type 1 IIR on a growing tumors, some studies with different tumor models were conducted in which therapeutical interventions were done to induce a shift of the IIR in growing tumors from a type 2 to a type 1 in ways different from those claimed in my patent application. In all cases where a shift from type 2 to type 1 was induced, the intervention caused tumor regression, Xiang et al. (J Interferon Cytokine Res. Vol. 20(4), pp. 349-54, 2000) **(see Table 1)**, Tsung et al. (J. Immunol. Vol. 160(3), pp. 1369-77, 1998), Winter et al. (J. Immunol. Vol. 163(8), pp. 4462-72, 1999), To et al. (The Laryngoscope, Vol. 110(10), pp. 1648-1654, 2000). In a different model of adoptive transfer of effector T cells expanded in vitro and then administered to mice, Winter et al. showed that specific effector T cells against the murine melanoma B16BL6-D5 that were transferred to animal growing D-5 derived pulmonary metastasis, induced complete regression of the metastasis as well as providing long-term immunity to D-5. Analysis of the therapeutic T cells showed that they exhibited a tumor-specific type 1 cytokine profile; secreting IFN-gamma but not IL-4, Winter et al. 1999. To et al., in a more elaborated model of adoptive transfer used effective T cells against a MCA 205 murine sarcoma, that were expanded in condition to become either with a Th1 phenotype or a Th2 phenotype. The activated expanded T cells with a Th1 lymphokine profile showed an excellent antitumor efficacy mediating tumor regression while the activated T cells expanded in the presence of IL-4 and anti-IFN-gamma antibodies had a Th2 lymphokine profile and demonstrated little antitumor efficacy, To et al. 2000. These preclinical studies demonstrate explicitly that the mechanism of tumor regression is a type 1 antitumoral inflammatory response.

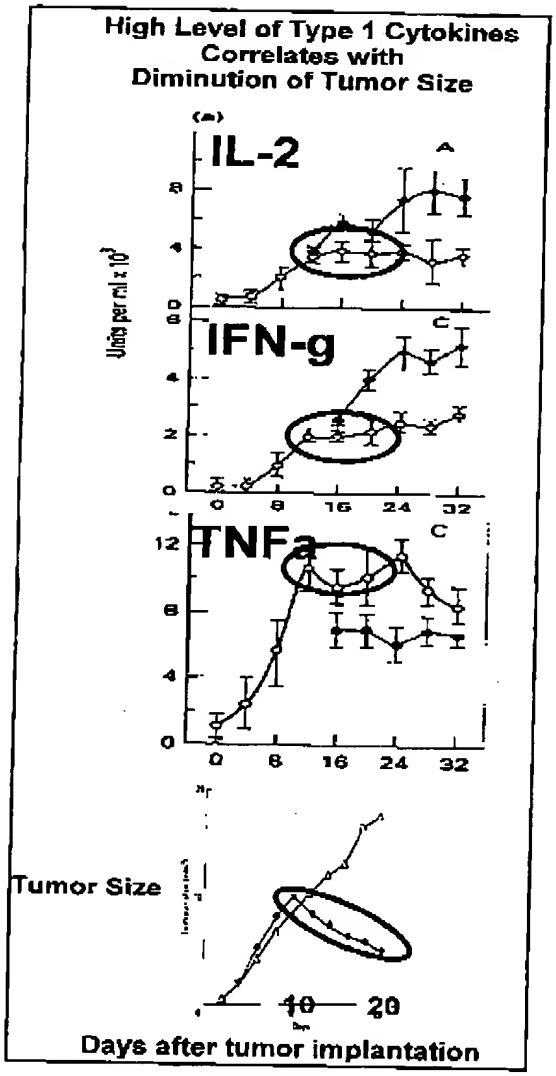

Figure 4

Analysis: The open circles correspondent to the level of the cytokines present in the regressing tumor tissue from day to day. The tumor size of the analyzed tissue (black losange) is plotted for the same days. When cytokines reach high levels the tumors begin to shrink.

4) The BCG treatment for human bladder cancer induces an intratumoral type 1 inflammation. It achieves complete remission in over 70% of patients.

Clinical proofs of the effectiveness of a type 1 inflammation to shrink tumors in a human patient exist already despite novelty of the field and they indicate unambiguously that an antitumoral type 1 IIR is the regression mechanism in human tumors.

1) Two different studies done by Halliday et al. comparing the intratumoral cytokines between spontaneously regressing and progressive primary melanomas and more recently between spontaneously regressing and progressive basal cell carcinomas showed that infiltrating T cells and the typel cytokines they produce, IFN-gamma, IL-2, and TNF-beta were elevated in both types of actively regressing tumors whereas no elevation of these same cytokines above controls was seen in both progressive tumors, Lowes et al. 1997, Wong et al. (British Journal of Dermatology Vol. 143, pp. 91-98, 2000). [This is one of the most compelling evidence of the power of the type 1 IIR to regress tumors in human. For some reasons, a few patients develop the ability to reset their immune defense and shrink their own tumors by shifting naturally their IIR from a type 2 to a type 1. The TMTIR will bring this possibility to all patients.]

2)Perhaps one of the most noticeable progress in cancer treatment for the past ten years is the discovery that the bacillus Calmette-Guerin (BCG) cause eradication of superficial bladder cancer in 70% of treated patients, Takashi et al. (Int. Urol. Nephrol. Vol. 30(6), pp.713-22, 1998), Jackson et al. (J. Urol. Vol. 159(3), pp.1054-63,1998), Lamm, (Urol. Clin. North. Am. Vol.19(3), pp. 573-80,1992). At least a dozen published studies report that the mechanism responsible of the tumor regression is the development of a local type 1 immune response (inflammatory response) against the bladder tumor. Clinical and laboratory evidences show that the antitumor activity is concentrated at the site of BCG administration indicating that local immune mechanisms are responsible for this phenomenon, Patard et al. (Urol. Res. Vol. 26(3), pp.155-59, 1998), Prescott et al. (Clin. Infect. Dis. Vol. 31 Suppl 3, pp.S91-3, 2000), Saint et al. (Prog. Urol. Vol. 10(6), pp.1118-26, 2000). The treatment consists of repetitive intra vesicular injections of live BCG bacillus that initiate and sustain a local inflammation. The local intra vesical immune response is intimately related to the interaction of three systems: the host (the patient), the BCG (mycobacteria) and the tumor. This interaction gives rise to a cascade of immunological events, some of which are essential to the protective action of BCG against relapse and tumor progression. The immune response to BCG is currently considered to comprise three phases. First of all, the BCG adheres to the urothelium and is then phagocytosed by antigen-presenting cells. This phase corresponds to early release of so-called inflammatory cytokines (IL1, IL6, IL8). The second phase consists of recognition of bacterial antigens by helper CD4 lymphocytes, which mainly release IL2 and IFNg. This cellular activation leads to the third phase: amplification of a cytotoxic populations of type 1 response capable of killing tumor cells: CD8 lymphocytes, macrophages, NK, LAK, and BAK cells shrinking the tumor completely. The end results of this complete antitumoral inflammatory response are tumor eradication and protection against relapses. Patard et al. 1998, Prescott et al. 2000, Saint et al. 2000.

TABLE 1. TUMOR GROWTH AND PROTECTIVE IMMUNITY AFTER INOCULATION OF VKCK/RM4-TNF-*a* AND VKCK/RM4-IFN-*g* TUMOR CELLS IN BALB/C MICE

	Tumor Incidence in mice (%)		
		Rechallenged with wild-type tumor b	
Tumor cells	*Primary injection* a	*VKCK*	*MCA-26*
VKCK/RM4-TNF-*a*	0/8 (0)	0/8 (0)	8/8 (100)
VKCK/RM4-IFN-*g*	1/8 (13)	0/8 (0)	8/8 (100)
VKCK	8/8 (100)	—	—
Control VKCK Irradiated c	0/8 (0)	8/8 (100)	—

a Relative number of tumor incidence in mice (%) after injection of VKCK/RM4-TNF-*a* ,VKCK/RM4-IFN-*g* , and VKCK tumor cells. Two weeks after tumor inoculation, tumors were removed and weighed. Tumor-free mice were confirmed after monitoring these mice for another 2 months.

b Mice with tumor regression of VKCK/RM4-TNF-*a* and VKCK/RM4-IFN-*g* tumor were rechallenged with 0.5 x 10(6) wild-type VKCK or MCA-26 tumor cells. Relative number of tumor incidence in mice (%) was calculated after rechallenge with wild-type VKCK or MCA-26 tumor cells.

Analysis: Animal model in which conditions were practiced to induce an intratumoral type 1 inflammatory response, Xiang et al. 2000 used a model of engineered tumor secreting cytokines in which three mouse tumors were used to elucidate the immune mechanisms for tumor regression versus tumor growth. These tumors were (1) the poorly immunogenic VKCK tumor (2) its derivative VKCK/RM4-TNF-a engineered to secrete tumor necrosis factor-alpha (TNF-alpha) and 3) VKCK/RM4-IFN-g engineered to secrete interferon-gamma (IFN-gamma) (see Table 1). The data showed that VKCK tumors grew aggressively in syngeneic BALB/c mice, and vaccination of irradiated VKCK cells failed to protect the mice from a subsequent challenge with the same tumor. In contrast, engineered VKCK tumor cells lost their tumorigenicity, and vaccination of engineered VKCK cells induced a protective immunity against VKCK cells that was mediated with VKCK-specific CD8+T cells. The T cell proliferative response and cytolytic activity against VKCK developed for all tumors aggressive and regressive, but in the aggressive ones these responses were much weaker compared with the regressive ones. The results indicated that regression of tumors engineered to secrete cytokines TNF-alpha or IFN-gamma was related to a shift from a host type 2 to a type 1 cytokine profile. Mice growing an aggressive tumor developed a type 2 response, whereas mice growing a regressing tumor developed a type 1 response to VKCK. Furthermore, the results indicated that the failure of unmodified VKCK to generate efficacious T cells was not due to an inability to recognize tumor antigens but rather, to the type 2 and magnitude of the antitumor immune response that developed.

Taken together these data indicate that tumors progress with an IIR type 2 and regress with an IIR type 1 in both animals and humans. The preclinical (animal) and clinical (human) evidences presented above demonstrate that therapies shifting intratumoral IR from type 2 to type 1 causes tumor regression. They represent firm proof-of- the- principles for the TMTIR and clear guidance asserting the alleviation of tumors in a human patient.